REMARKS

Claims 40-44 and 51-68 are pending in the present case. Claims 40-44 and 51-68 stand rejected under 35 U.S.C. § 112, first and second paragraphs. Each of these rejections is addressed below.

Cancellation of Non-Elected Claims

In the current Office Action, the Examiner indicates that non-elected claims 1-39 and 45-50, although withdrawn, remain pending in the application and should be canceled. These claims are hereby canceled without prejudice.

Rejection of claims 40-44 and 51-68 under 35 U.S.C. § 112, second paragraph

Claims 40-44 and 51-68 stand rejected under 35 U.S.C. § 112, second paragraph, for indefiniteness. The Examiner states that the rejection based on Figure 3 of Mead et al. is maintained and that Dickinson et al. leaves open the possibility that there are additional known sequences for the tenth module of the human fibronectin type III domain.

Applicants first note that the 35 U.S.C. § 112, second paragraph rejection cited in the previous Office Action referred to Figure 3 of Main et al. (*Cell* 71:671-678, 1992) (not of Mead et al.), and Applicants have assumed that the Examiner is in fact referring to this same Main et al. Figure 3 in making the present rejection.

With respect to the substance of the rejection, Applicants submit that the reliance

on Figure 3 of Main et al. for support for the indefiniteness rejection is incorrect, as it is based on a misinterpretation of this Figure. Main Figure 3 does not indicate that there are multiple sequences for the *tenth module* of human fibronectin type III, but rather shows that there are sixteen known *modules* of type III fibronectin that make up this protein (i.e., "F1"-"F16"), all of which share some degree of relatedness. These sequences therefore do not represent many different sequences for the tenth module of human fibronectin type III protein, but instead represent *sequences for many different modules* of the human fibronectin type III protein.

The sequence for the *tenth module* of human fibronectin type III disclosed in Main et al. is the same as that disclosed in the present application at Figure 4. In addition, this sequence is the same as the "sequence encoding the structure of the ^{10}Fn3 domain referred to as "1ttg" (ID="1ttg" (one ttg)) available from the Protein Data Base," described on page 8, lines 16-18 of Applicants' specification. Clearly, there is only one known sequence for the tenth module of human fibronectin type III and this same sequence is found in Main et al., in the Protein Data Base, and in the specification and drawings of the present application.

The Examiner also refutes Applicants' use of Dickinson et al. as further support for the one known amino acid sequence of the tenth module of human fibronectin type III by citing section (g) as evidence that Dickinson leaves open the possibility of intra modular interactions which would lead to uncertainty or variability in the module's

sequence. Applicants respectfully disagree.

In section (g), Dickinson discusses the possibility of using the crystal structure of the tenth module of human fibronectin type III to predict the structure of any module of human fibronectin type III, *other than the tenth module*, where the structure is not known. Structural alignment based on sequence comparisons is commonly used to predict protein structure for homologous proteins or protein modules. In this section, Dickinson refers to the *structural variability* that can be expected for those modules having loops which differ in sequence and length. Dickinson does not refer to any type of sequence variability that occurs in the tenth module itself.

In addition, the reference to "intra modular interactions" again relates to structural variability and not sequence variability. Dickinson states, "Since connecting segments protrude from the ends of the III_{10} module, sequence in these loops are accessible for intra- or inter-molecular interactions, and thus the *structural variability* in loops may contribute to the distinct functional roles of the FNIII modules...(emphasis added)." Again Dickinson is referring to the structural variability in the loops of the various modules of human fibronectin type III. Dickinson does not suggest that there is sequence variability or that alternative sequences exist for the tenth module of human fibronectin type III. Accordingly Applicants assert that there is only one known sequence and that the claims are definite in their recitation of the "*tenth module* of human fibronectin type III (^{10}Fn3)."

While Applicants disagree with the present rejection based on the arguments outlined above, independent claims 40, 41, and 59 have been amended to include the amino acid sequence of the tenth module of human fibronection type III as listed in Figure 4 and on page 8, lines 16-18 of the specification. Given the addition of this sequence to the claims, there can be no possible ambiguity, and this rejection should be withdrawn.

Claim 59 also remains rejected based on the assertion that the claim is indefinite as to which amino acid alteration(s) constitute the invention.

As described on page 9, lines 1-2 of the specification, randomized includes one or more alterations to the amino acid sequence relative to the template sequence (in this case, the tenth module of human fibronectin type III). It is precisely this "randomization" that allows for an optimization of the number of distinct scaffold-based proteins available to carry out the methods of claim 59.

The specification provides clear guidance as to which amino acids should be randomized relative to the template sequence. For example, on page 17, line 9 to page 18, line 11, the specification states (emphasis added):

> In addition, we have determined that the ^{10}Fn3 framework possesses exposed loop sequences tolerant of randomization, facilitating the generation of diverse pools of antibody mimics. This determination was made by examining the flexibility of the ^{10}Fn3 sequence. In particular, the human ^{10}Fn3 sequence was aligned with the sequences of fibronectins from other sources as well as sequences of related proteins (Figure 4), and the

> results of this alignment were mapped onto the three-dimensional structure of the human ^{10}Fn3 domain (Figure 5). This alignment revealed that the majority of conserved residues are found in the core of the beta sheet sandwich, whereas the highly variable residues are located along the edges of the beta sheets, including the N- and C-termini, on the solvent-accessible faces of both beta sheets, and *on three solvent-accessible loops* that serve as the hypervariable loops for affinity maturation of the antibody mimics. In view of these results, the randomization of these three loops are unlikely to have an adverse effect on the overall fold or stability of the ^{10}Fn3 framework itself.
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> For the human ^{10}Fn3 sequence, this analysis indicates that, at a minimum, amino acids 1-9, 44-50, 61-54, 82-94 (edges of beta sheets); 19, 21, 30-46 (even), 79-65 (odd) (solvent-accessible faces of both beta sheets); 21-31, 51-56, 76-88 (CDR-like solvent-accessible loops); and 14-16 and 36-45 (other solvent-accessible loops and beta turns) may be randomized to evolve new or improved compound-binding proteins. In addition, as discussed above, alterations in the lengths of one or more solvent exposed loops may also be included in such directed evolution methods. Alternatively, changes in the β-sheet sequences may also be used to evolve new proteins. These mutations change the scaffold and thereby indirectly alter loop structure(s). If this approach is taken, mutations should not saturate the sequence, but rather few mutations should be introduced. Preferably, no more than 10 amino acid changes, and, more preferably, no more than 3 amino acid changes should be introduced to the β-sheet sequences by this approach.

In this exemplary section, the specification clearly describes which amino acid residues are to be randomized to generate the scaffold-based proteins provided in claim 59. Therefore, claim 59 is definite in that it refers to randomization in the specific loops of the tenth module of human fibronectin type III described in the specification. Applicants assert that this claim is indeed definite with regard to "which amino acid alterations constitute the invention," and this rejection may be withdrawn.

Rejection of claims 40-44 and 51-68 under 35 U.S.C. § 112, first paragraph

Claims 40-44 and 51-68 stand rejected under 35 U.S.C. § 112, first paragraph, for failure to convey possession of the claimed invention at the time the application was filed. The Examiner has asked Applicants to point out, in the specification, the location of any "three randomized loops" on a scaffold-based protein and evidence of the binding of any "three randomized loops" to a compound not bound by the human ^{10}Fn3 sequence.

In response, Applicants first note that the specification indicates in a number of locations that all three of the human ^{10}Fn3 loops may be randomized to produce the scaffold-based proteins of the invention. For example, on page 15, line 18 to page 16, line 8, the specification states:

> The three loops of ^{10}Fn3 corresponding to the antigen-binding loops of the IgG heavy chain run between amino acid residues 21-31, 51-56, and 76-88 (Figure 3). The length of the first and the third loop, 11 and 12 residues, respectively, fall within the range of the corresponding antigen-recognition loops found in antibody heavy chains, that is, 10-12 and 3-25 residues, respectively. Accordingly, once randomized and selected for high antigen affinity, these two loops make contacts with antigens equivalent to the contacts of the corresponding loops in antibodies.
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> In contrast, the second loop of ^{10}Fn3 is only 6 residues long, whereas the corresponding loop in antibody heavy chains ranges from 16-19 residues. To optimize antigen binding, therefore, the second loop of ^{10}Fn3 is preferably extended by 10-13 residues (in addition to being randomized) to obtain the greatest possible flexibility and affinity in antigen binding. Indeed, in general, the lengths as well as the sequences of the CDR-like loops of the antibody mimics may be randomized during in vitro or in vivo affinity maturation (as described in more detail below).

Additional descriptions of preferred fibronectin-based scaffolds can be found on page 4, lines 6-12, and in the following excerpt from page 17, lines 9-22, which states (emphasis added):

> In addition, we have determined that the ^{10}Fn3 framework possesses exposed loop sequences tolerant of randomization, facilitating the generation of diverse pools of antibody mimics. This determination was made by examining the flexibility of the ^{10}Fn3 sequence. In particular, the human ^{10}Fn3 sequence was aligned with the sequences of fibronectins from other sources as well as sequences of related proteins (Figure 4), and the results of this alignment were mapped onto the three-dimensional structure of the human ^{10}Fn3 domain (Figure 5). This alignment revealed that the majority of conserved residues are found in the core of the beta sheet sandwich, whereas the highly variable residues are located along the edges of the beta sheets, including the N- and C-termini, on the solvent-accessible faces of both beta sheets, and *on three solvent-accessible loops* that serve as the hypervariable loops for affinity maturation of the antibody mimics. In view of these results, *the randomization of these three loops* are unlikely to have an adverse effect on the overall fold or stability of the ^{10}Fn3 framework itself.

These excerpts from the specification indicate that the scaffold-based proteins of the invention may indeed include "three randomized loops."

Moreover, detailed descriptions of the production of exemplary libraries that include ^{10}Fn3-based proteins having three randomized loops and the use of these libraries for identifying proteins that are not bound by the tenth module of human fibronectin are also found in the specification. In particular, at page 22, line 22 to page 28, line 9, Applicants describe the production of a library of ^{10}Fn3-based proteins that include three randomized loops. This library was created by randomizing the coding sequences for

each of the BC, DE, and FG loops separately, and then combining fragments encompassing these randomized loops to generate final nucleic acid sequences that encode scaffold proteins possessing mutations in all three loops.

Using a library that included these proteins, Applicants carried out a screen against a target not bound by human ^{10}Fn3 – specifically, against the human TNF-α protein. On this point, the Examiner is directed to the specification at page 36, line 22 to page 38, line 8, where a selection using TNF-α and a library of human ^{10}Fn3 variants with randomized BC, DE, and FG loops is described as follows:

> In one exemplary use for fibronectin scaffold selection on chips, an ^{10}Fn3-based *selection was performed against TNF-α, using a library of human ^{10}Fn3 variants with randomized loops BC, DE, and FG.* The library was constructed from three DNA fragments, each of which contained nucleotide sequences that encoded approximately one third of human ^{10}Fn3, including one of the randomized loops. The DNA sequences that encoded the loop residues listed above were rebuilt by oligonucleotide synthesis, so that the codons for the residues of interest were replaced by (NNS)n, where N represents any of the four deoxyribonucleotides (A, C, G, or T), and S represents either C or G. The C-terminus of each fragment contained the sequence for the FLAG purification tag.
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> Once extended by Klenow, each DNA fragment was transcribed, ligated to a puromycin-containing DNA linker, and translated in vitro, as described by Szostak et al. (Roberts and Szostak, Proc. Natl. Acad. Sci USA 94:12297, 1997; Szostak et al., U.S.S.N. 09/007,005 and U.S.S.N. 09/247,190; Szostak et al., WO98/31700), to generate an mRNA-peptide fusion, which was then reverse-transcribed into a DNA-mRNA-peptide fusion. The binding of the FLAG-tagged peptide to M2 agarose separated full-length fusion molecules from those containing frameshifts or superfluous stop codons; the DNA associated with the purified full-length fusion was amplified by PCR, then the three DNA fragments were cut by Ear I restriction endonuclease and ligated to form the full length template. The template was transcribed, ligated to puromycin-containing DNA linkers, and translated to generate a ^{10}Fn3-PROfusion™ library, which was

then reverse-transcribed to yield the DNA-mRNA-peptide fusion library which was subsequently used in the selection.

Selection for TNF-α binders took place in 50 mM HEPES, pH 7.4, 0.02% Triton-X, 0.1 mg/mL salmon sperm DNA. The PROfusion™ library was incubated with Sepharose-immobilized TNF-α; after washing, the DNA associated with the tightest binders was eluted with 0.1 M KOH, amplified by PCR, and transcribed, ligated, translated, and reverse-transcribed into the starting material for the next round of selection.

Ten rounds of such selection were performed (as shown in Figure 13); they resulted in a PROfusion™ pool that bound to TNF-α-Sepharose with the apparent average Kd of 120 nM. Specific clonal components of the pool that were characterized showed TNF-α binding in the range of 50-500 nM.

Thus, as indicated, a library of proteins with three randomized loops was generated and used to select TNF-α binders. Following ten rounds of such selection, pools of proteins that bound to TNF-α with an apparent average Kd of 120 nM, as well as specific proteins within those pools, were successfully obtained.

Based on the above, Applicants submit that they have provided the locations, within the specification, of "three randomized loops" on a scaffold-based protein as well as evidence of the binding of a protein having "three randomized loops" to a compound not bound by the human ^{10}Fn3 sequence. In so doing, Applicants have satisfied the Examiner's request, and the § 112, first paragraph rejection should be withdrawn.

CONCLUSION

Applicants submit that this case is now in condition for allowance, and such action is respectfully requested.

Enclosed is a Notice of Appeal to the Board of Patent Appeals and Interferences appealing the Examiner's decision finally rejecting claims 40-44 and 51-68. In addition, a petition to extend the period for replying for three months, to and including September 11, 2003, and a check in payment of the required extension fee are also enclosed.

If there are any additional charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

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